F. H. C. Crick, F.R.S.

Kieckhefer Distinguished Research Professor, The Salk Institute, San Diego, California, U.S.A.

Awarded the Nobel Prize for Medicine (jointly) in 1962, the Royal Medal of the Royal Society in 1972 and the Copley Medal in 1975. Honorary Fellow of Churchill College and Caius College, Cambridge. Fellow of University College, London.

DEVELOPMENTAL BIOLOGY

Our ignorance of developmental biology has the following curious feature. We understand how an organism can build molecules (even very large molecules) in great variety and with great precision, although the largest of them is far too minute for us to see, even with a high-powered microscope; yet we do not understand how it builds a flower or a hand or an eye, all of which are plainly visible to us. We understand much of what goes on inside a small cell, such as a bacterial cell, whose dimensions are no more than a few times the wavelength of visible light, yet we are ignorant of many important processes in our own cells, and indeed in the cells of most animals and plants. How these cells unite together to form tissues, the tissues to form organs, and the organs to form the whole organism, we can describe but we cannot yet fully comprehend. True, much can be followed with the optical microscope, which allows us to see cells, to see when they divide and when and where they move, but this is not enough. The reason is simple. Life is engineered at the molecular level. To explain what we see, we must understand what we cannot see. And thus, paradoxically, the nearer we are to atoms the more easily we can grasp what is really happening, in spite of the formidable experimental difficulties.

The aim of developmental biology is to explain as fully as possible how an egg and a sperm are made, how they come together to form the fertilized egg, how the cell divides and divides again and again to form a small hollow ball of cells — the blastula; how this undergoes a complicated series of internal movements to form the gastrula, how the various cells change their shape and character to form tissues — and so on until the mature animal itself is built. But developmental biology is wider than this, since it encompasses plants as well as animals, both the familiar higher organisms like the apple tree and the giraffe, as well as those more lowly and less familiar such as the sea urchin and the slime mould.

To appreciate what we yet have to discover, we must first outline what we already know, and this is no easy task since our knowledge has grown enormously in the present century, and especially during the last 25 years. Leaving aside most of the hard parts, living organisms are mainly made from organic molecules, and most of these molecules the organism must synthesize for itself. The raw materials are obtained in various ways. In plants by using photosynthesis to fix the carbon dioxide in the air and from salts taken up by the roots from the soil. In animals by breaking down food into small molecules which are then used as building blocks or as a source of energy. Each simple step of chemical synthesis, typically turning one small molecule into a closely related one, is catalysed by a special catalyst, specific for that step alone, and each of these catalysts (or enzymes, as they are called) is a *large* chemical molecule, typically having tens of thousands of atoms, of the family known as protein. That is, it is made by stringing together certain smaller molecules into long chains. Of the smaller molecules (known as amino acids) there are twenty different types used to build protein and it is the precise order in which they are strung together which makes a particular protein what it is. Moreover, since proteins are made with great precision and are, as a family, both versatile and subtle in their chemical properties, they are used for other important functions: to build structures, to act as signals and so on. A small bacterial cell may possess several thousand *types* of protein, our own cells rather more. Not surprisingly different sets of cells have somewhat different batteries of proteins. A muscle cell has many special proteins needed to construct the contractile apparatus. A red blood cell is stuffed with the protein haemoglobin, which is an oxygen carrier. To a large extent, a cell is what it is because of its proteins. Thus we need to know how proteins are synthesized and how each type of cell produces just the proteins it requires.

Here again, our knowledge has grown enormously. The production of each protein is

controlled by a different gene. The essential part of each gene is made of nucleic acid which acts as a molecular message, specifying with great precision the amino acid sequence of that particular protein. We know how working copies are made of this message, how they are read by special reading heads (called ribosomes) each an assembly of large molecules. We know the genetic code; that is, the dictionary which relates the four-letter language of the nucleic acid to the twenty-letter language of the protein. And in addition we know how genes are copied so that each daughter cell may have an exact replica of the genetic instructions stored in its parent. Such, in the briefest outline, is our understanding of the fundamental processes of gene structure, gene replication and gene action.

The first major gap in our knowledge is that while we understand a gene in a very simple organism, such as a bacterial cell, we are uncertain exactly what is in a higher organism. Our chromosomes appear to have much more DNA (deoxyribonucleic acid) than we would expect. Also they have much more protein associated with them than we find associated with bacterial chromosomes. The primary gene product appears too large for a simple messenger function and much of it is broken down rather quickly and never leaves the nucleus of the cell — why, we do not yet know. Nor do we have any example of how, in detail, genes are controlled in higher organisms. However, we are sure that, as in bacteria, proteins are coded by sequences in the DNA of the chromosomes, so we think we understand the essential features of all these processes. It is the baroque superstructure which has so far eluded us; but this is an area of intensive research and one can reasonably expect that the picture will clear dramatically within the next 5 or 10 years.

There are other aspects of the cell which we now understand much better than we did even 5 years ago. Every cell is bounded by a membrane which acts as a barrier to molecules on the outside getting in and, equally important, to many small molecules on the inside which would otherwise leak out. Special proteins act as gates across this external membrane, allowing desirable small molecules to be pumped into the cell, and various unwanted molecules to leave. There are also transducer proteins which, when activated by special chemical signals on the outside, will produce a chemical change inside the cell. In addition, there are membranes *inside* the cell — the membrane of the nucleus is one example — and there are devices whereby packets of molecules, wrapped in a small piece of membrane, can be released from the cell.

Basically a membrane is made of a lipid bilayer which has hydrophobic (water-hating) groups on its inside and hydrophilic (water-loving) groups on its two outer sides. We now know that the bilayer is fairly fluid and we are beginning to understand something about the proteins in it. We need to know much more, especially how the components of the membrane are synthesized and assembled together. Again this is a very active field of research.

Within each cell molecules and organelles are moved about. Cells can change shape, divide, send out processes. How are these movement produced? The molecular tools used appear to be fibrillar protein molecules, long thin aggregates of molecules assembled from protein subunits. We know four major types of these: two, actin and myosin, are closely related to the two major proteins in muscle which slide over each other to produce muscular contraction; one, tubulin, is also found, with other proteins, as the basic structural element in the cilia and the flagella of higher organisms; and one, the protein of neurofilaments, is only just being characterised. There are probably others — certainly other molecules found in muscles are also found in ordinary cells — but it is possible that these four are the only major factors as far as cell movement is concerned. They occur in many animal cells but we still do not know if they are all present in plants. (Plant cells usually have rigid walls and thus move much less than animal cells, although their cytoplasm often flows rapidly within the cell.) Again, we may expect rapid progress in

identifying these molecules, locating them in the cell, and finding how they interact. There is one major unsolved problem. How do these fibrillar elements, which produce movement, themselves get shunted to the right place in the cell, at the right time, to produce just the right movement? For example, how does the mitotic spindle form in the correct orientation? And how does a muscle fibre assemble all its components to produce a highly ordered contractile machine? The answer may come from studies on the fibrillar molecules themselves and how they interact or it may involve some other principle. Here we know so little we can only wait and see what will turn up.

But by far the largest area of our ignorance is not what happens inside a cell but what happens between cells. Here our knowledge is fragmentary, since what we know is quite inadequate to explain what we see. Each cell in a multicellular organism is obviously influenced by the general chemical environment in which it is bathed. It must be supplied with sugars, amino acids and other small molecules it needs, since our own cells are not as versatile as many bacterial cells and, not being able to synthesize some of these small molecules for themselves, are dependent upon an external supply. But in addition there are specific chemicals which are in effect instructions. It is not their metabolism that matters, but the controlling effects they have on many sets of metabolic processes inside cells. Many such molecules are known — the sex hormones are good examples of this — and something is being learnt about how they act at the molecular level. But the interactions of cells are regulated in other less familiar ways. In a sheet of cells — an epithelium — a cell can often be shown to know, in some way, where it is in the sheet. It has what is called "positional information", as can be demonstrated in special cases by moving it somewhere else in the sheet and seeing how it behaves. It also knows which way round it is in the sheet. For example, if it produces a bristle, that bristle will often lean over in a particular direction. Cells are probably influenced quite strongly and specifically by their contacts with neighbouring cells. We can see that they often form special junctions, of several different types, when they come into contact and we know some molecules can move from cell to cell across some of the junctions. It is a measure of our ignorance that we do not know whether "positional information" is mediated by small molecules, by cell to cell contact, or by some other process such as signals, analogous in a loose sense to the signals passing down a nerve cell, passing over the tissue. Then again, for a tissue to grow to a fixed size and shape, there must be some control of the divisions of its cells and in many cases over the direction of these divisions. Here again, when we realise what a complicated job has to be done, we can see that our ignorance far exceeds our knowledge. At least we know that it is not always done by a strict lineage mechanism. In some simple organisms — for example a soil nematode — it has been shown that a particular cell in a developing animal will always produce exactly the same descendents. It will divide twice; one of the four grand-daughters will always die and the other three each develop in a special way. If this mother cell is killed in early development, all the three special grand-daughter cells will be absent. No other cell will take over the function of the dead cell. But in other organisms — the fruit fly, for example — killing one cell of a group, at an early stage in development, will merely mean that the others divide rather more often so that in the adult the final number of descendants of that group will be roughly the same as usual.

Perhaps the most challenging problem in the whole of developmental biology is the construction of the nervous system of an animal. Many years ago it was shown by Sperry that if a newt's eye was removed, so that the optic nerve from its eye to its brain was broken, then even if the eye was replaced upside down the optic nerve would regenerate from the retina, grow towards the brain and connect up again. After a period the animal could see again with this eye but it always saw upside-down (newts not being clever enough to adjust to such a

situation). In other words the new connections had been made "correctly" except that the eye did not know that it had been inverted. Such experiments, and many sophisticated variants of them, have been repeated in recent years by several groups of workers. All their results show that fairly precise processes are at work to make the correct, rather intricate, connections needed between one set of nerves and another but exactly what these mechanisms are we do not yet know. We do not even know just how precise they need to be.

I have spoken as if all these problems were the same in all multicellular organisms but this ignores the immense variety of Nature. To a biologist an elephant and a field mouse are very similar — they are both mammals. It would be surprising if in these two cases the general problems of development did not have similar answers. Again one might guess that the other vertebrates — the fishes, the amphibia, the reptiles, the birds — would not be very different. But what about the higher invertebrates such as the octopus and the squid, or the vast array of insects, to say nothing of various worms, jellyfish, sponges . . . right down to the amoebas. Are all these unknown processes likely to be just the same in plants as in animals? And will there be major differences between the flowering plants and the lower members of the plant kingdom such as the fungi? We can only guess at the answers. Because living organisms look so different it does not mean that their basic biochemical mechanisms are not the same. The great families of the nucleic acids and the proteins are put together in the same general way in all organisms. Even though individual proteins usually differ somewhat in different species they are often remarkably similar — not surprisingly since they have usually evolved from a common ancestor. The general uniformity of biochemistry throughout Nature is one of the most astonishing discoveries of this century. But we must not press it too far. The hormones of plants are very different from those of animals. Moreover, plants and animals are, to some extent, built on different principles. On a broad scale a tree is only statistically defined. The exact branching pattern can be very different from one tree to another, even if they are identical genetically. On this scale a higher animal is made with greater precision — two eyes, four legs and so on. But then the flowers of a particular plant are often constructred rather precisely while, on the other hand, certain tissues in animals — for example the branching patterns of the bronchioles in the lungs — appear as loosely defined as the branches of a tree. Certainly there must be a variety of mechanisms at work. Some are likely to be the same, or very similar, throughout Nature; others may be less widely distributed.

Developmental biology, then, is an area of the greatest biological importance where our ignorance is more striking than our knowledge. At the visible level, as seen in the optical microscope, we can describe an organism in terms of its cells — itself a major advance over the knowledge of earlier times. In many cases we can see these cells grow, divide and change shape. At the molecular level we understand some of the most fundamental biochemical processes and we are rapidly extending our knowledge. But how an organism constructs a hand, with its thumb and four fingers, with all the bones, the muscles and the nerves, all assembled and correctly connected together, that we cannot yet explain with the force and finality which characterises a successful piece of science. We have a profound and reasoned belief that it can be done, but it will take some time, much hard work and no little imagination to do it successfully.